Characterization of the Major Tail Protein gpP encoded by *Lactobacillus plantarum* phage φg1e

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Occurrence of lysogeny among abundant lactobacilli has been recognized widely. Like *Escherichia coli* lambda system [1], the *Lactobacillus* temperate phages have been regarded as valuable genetic tools for gene transfer and cloning, in these industrially and medically important genera

In contrast to coliphages such as lambda [1], molecular details of the *Lactobacillus* phages are still unclear in genome structure, gene expression, host range, replication, classification, evolution, and so on. Recently, we have isolated a new *Lactobacillus plantarum* temperate phage φg1e, and determined the total genome sequence of 42259-bp DNA (EMBL accession number X98106) [2].

φg1e significantly differs from other *Lactobacillus* phages in gene structure as well as morphology.

As reported previously [3], the φg1e phage particle contains four major proteins, gpB, gpG, gpO, and gpP (64, 43, 29, and 26 kDa, respectively), as well as more than 16 minor proteins ranging from 113 to 9.6 kDa, and consists of three parts: (i) an isometric hexagonal head (68 nm in diameter), (ii) a long noncontractile-flexible tail (256 nm in length and 10 nm in width) with regularly spaced transversal striations, and (iii) a complicated stacked-baseplate (from 24 to 45 nm in diameter) ending in several short fibers (12 nm long). The protein gpP (as well as gpG) was acidic, and its apparent molecular mass on SDS polyacrylamide gel was 26.0 kDa, significantly larger than that (18.8 kDa) predicted from the DNA sequence [2].

On prolonged preservation at 4℃ in Tris-based phage buffer, the φg1e particles lost biological ability for making clear zones [3]. These preserved particles contained electrophoretically variant gpP proteins (as well as gpG), which migrated faster on SDS gel than those of the fresh proteins. When preserved for 5 months (Fig. 2 lane 3), the altered gpP yielded an apparent molecular mass of 18.8 kDa, which was substantially lower than the value (26.0 kDa) of the fresh gpP, and corresponded well with that (18.8 kDa) predicted from the DNA sequence [2]. At one-month preservation, the phage particles still retained the biological ability, though producing turbid zones, and their gpP proteins migrated faster on SDS gel than those of the fresh sample, but slower than those of the 5-months preserved proteins. Furthermore, electron microscopic examinations demonstrated that the base plates of the 5-months particles were located along with the tail, but not at the tip, seemingly slipping out of the

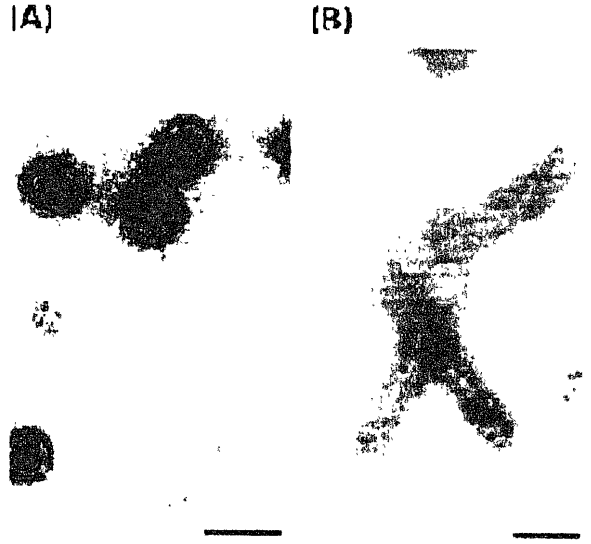

Fig. 1. Immunoelectron micrographs of ϕ gle virion particles. Φ gle particles were purified by CsCl centrifugation, and examined by immunoelectron microscopy. (A) Non-labeled particles. (B) Gold-labeled particles. Bars: 100nm.

tip toward the head. This base plate dislocation was not detected in the phage preparation preserved for one month. To elucidate whether the electrophoretical abnormality of the gpP protein is linked to the base-plate movement, several properties of this protein have been further characterized.

To overproduce gpP, the *P* gene encoding 177 amino acid residues was cloned under *plac* of *E. coli* plasmid pUC118. As shown in Fig. 1, a 679-bp *Hha*I fragment of the ϕ gle genome (42259-bp: EMBL accession number X98106) contains gene *P* and its putative SD sequence as well as truncated genes *Rorf135* and *Rorf143* [2]. This *Hha*I fragment was blunted, and introduced into the *Sma*I site of pUC118, yielding p118P. DNA sequencing analysis confirmed that p118P contained the intact *Hha*I fragment from nucleotide number 21923 to 21247 [3]. The plasmid p118P could multiply stably at 37℃ in *E. coli* XL1-Blue.

After induction by isopropyl β-D-thiogalactopyranoside (IPTG) (1 mM) at 37℃, total proteins were extracted from *E. coli* XL1-Blue/p118P, and subjected to SDS-PAGE. The p118P directed steadily increasing synthesis of a protein with an apparent molecular mass of 26.0 kDa, which was not detected in *E. coli* XL1-Blue/pUC118 (Fig. 3A). This overproduced protein had an apparent molecular mass and N-terminal amino acids identical to those of the native-gpP protein from the purified ϕ gle particles [3]. These results indicate that gpP overproduced in *E. coli* is the gene *P* protein. To know localization of gpP in the ϕ gle particle, gpP was purified by DEAE-cellulose column chromatography and gpP-specific antibodies were prepared using the purified-gpP proteins. The analysis by immunoelectron microscopy demonstrated that immunogold particles associated with antigpP-sera specifically bound to the tails of ϕ gle particles (Fig. 1), indicating that gpP is a main tail component (putatively a tube protein).

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